

## COMPARATIVE STUDY OF AROMA CHEMICALS FROM *MENTHAPIPERITA* VIA: MICROWAVE ASSISTED HYDRODISTILLATION, SOLVENT FREE MICROWAVE EXTRACTION AND HYDRODISTILLATION PROCESSES

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### ABSTRACT

*Menthapiperita* is one of the important traditional medicinal plants widely used in pharmaceuticals, cosmetic, food and flavor industries and belongs to the labiatae family. The plant in this family is widely used to improve concentration and memory level. Altering the extraction methods of economically important mentha oil may allow raising the engineer composition, increasing the rate of oil's extraction with lesser energy demand. The experiment was performed to model higher mentha oil yield as the function of distinct operating parameter. The intent of this work was to differentiate the three extraction method- Solvent free extraction method, Microwave assisted hydro distillation and hydro distillation in term of high yield of peppermint oil with less energy consumption. This experiment was designed to evaluate the interaction of significant parameters such as solvent to raw material ratio, extraction time and microwave power on the yield of mentha piperita oil. The material used in this work includes the fresh leaves of mentha piperita. The extractions by microwave assisted hydro distillation were done on the power of 200W, 250W and 300W; the ratio of solvent and raw material to be extracted (4:1, 6:1, 8:1, 10:1) and extraction time was 30, 60, 90 and 120 min. whereas extraction by SFME method were done on same power level at constant load of plant matter in the same level of extraction time. From the results, the maximum yield of peppermint oil was achieved from solvent free microwave extraction method with 1.02% yield at 250W in 90min. And SFME method was discovered to be the best method in the term of yield as it compared with other two methods 0.98% of yield in 90 min from MAHD method and 0.97% of yield from HD after 4.5 hour of distillation time). Therefore, SFME is considered to be the new green technology for extracting oil as a more energy saver, money saver and clean process. Testing of volatile compounds present in extracted peppermint oil was investigated by Gas Chromatography and mass spectrometry. And testing has confirmed that there is no effect of extraction technique on the main constituents of oil as the content of menthol compound was noticeably same. From the experimental work SFME proved as a superior and green method for the extraction of higher essentials oil with lesser energy required.

**KEYWORDS:** *Menthapiperita*, MAHD, SFME, HD & GC-MS

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### INTRODUCTION

Essential oils concentrated horticultural extract of distinct aromatic plant species that emanate from seeds, flowers, leaves, wood and bark, but also from roots, fruits and various additional plant materials, also known by fly oil. It is enumerated that there are 3,00,000 approximately number of plants worldwide and essential oil is present in about 10% and could be used as a source for their production (Husnu et. al. 2010). Despite the quantity of essential oil in the plant elements is very squat (mainly 1-8% of whole mass of the plant), that is why, it is extremely necessary to

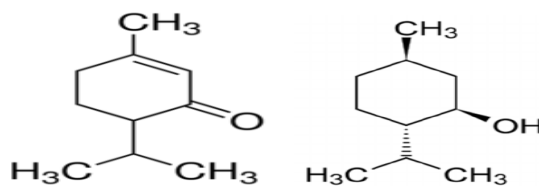
optimize the extraction technique parameters in order to attain a maximum yield and valuable quality oil (Bradley et. al. 1992). Their balmy quality enables them to be used as flavoring in both the food and beverage industries. These oils are also extensively used in both the cosmetic and pharmaceutical industries. The chemical composition of the essential oils is important as it is used in determining their quality and price in the market. From clinical studies, it is proved that higher frequency matter destroyed the disease of lower frequency and essential oil is the oil with highest frequency substance that known to human.



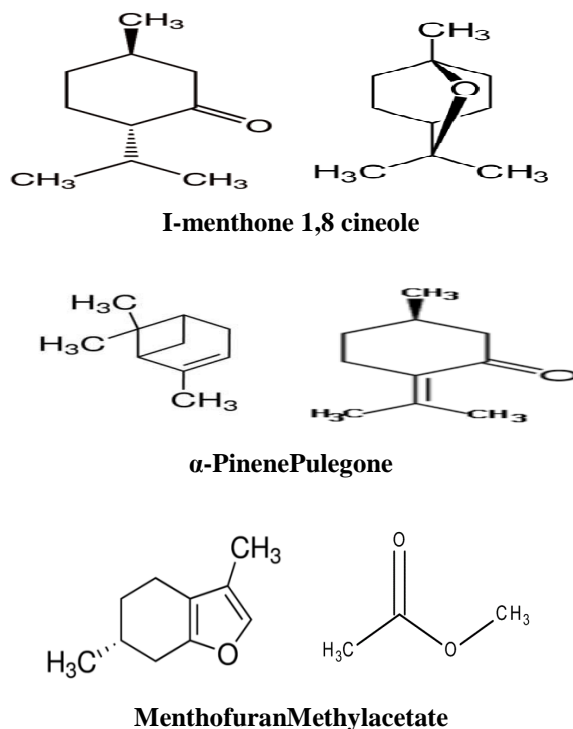
**Figure 1: Menthapiperita Leaves**

The genus *Menthapiperita* (Peppermint) a medicinally important plant is the member of Labiatae (Lamiaceae) group. Since ancient time, Europe, Africa, Asia, Australia and North America are the clement areas in the world for this species[Gavahian et. al. 2015]. The plant is native to Europe and is now widely cultured all over the world [Jianming et. al. 2010]. In India, Uttar Pradesh, Uttarakhand, Himachal Pradesh, Jammu and Kashmir are the states of large cultivation area of mentha piperita plant. 80% of total world demand fulfills by India with 8,000 tons per year production. The genus *Mentha* includes many Species, Varieties and hybrids. This includes 25-30 species approximately. Three of them- *Mentha arvensis* L., *menthaspicata* L., and *mentha piperita* L. have been approved for International marketing by ISO (Dashora et. al. 2006). The peppermint leaves are in shape of quadrangle with serrated edges of 50-90 cm high. The relative concentrations of this plant can vary with plant maturity, on climate, cultivar, and geographic location. Extracted *M. piperita* oil contain some active ingredients, such as menthol, menthone together with other chemical constituents including menthofuran, menthyl acetate, 1,8-cineole, b-pinene, a-pinene, isomenthone, and limonene. The usefulness of this oil is for depression and mental fatigue and for refreshing the spirit, improving the concentration level and in pharmaceuticals industry.

#### Structure of Component of Peppermint Oil



**Piperitone Menthol**



Numerous traditional techniques have been used to produce volatile and non-volatile essential oil—steam distillation, water distillation, soxhlet extraction, empyreumatic distillation, maceration, supercritical fluid extraction, hydrodistillation etc. However, due to some drawbacks of these methods limit its utilization.

**Table 1: Advantages and Disadvantages of Different Technique**

Extraction method	Advantage	Disadvantage	Reference
Steam Distillation	Easy to operate, low extraction cost, no- solvent involvement	Low extraction efficiency, time consuming process, require lot of energy	Dai et. al. (2010), Chen et. al. (2015)
Soxhlet extraction	Not necessarily require high temperature	Quality oil lost during solvent evaporation, high extraction time	Dai et. al. (2010)
Supercritical CO <sub>2</sub> extraction	Less extraction time, enhance quality of oil	Operational complexity due to high pressure and not cost effective, not available in routine library	Golmakani et. al. (2008), Chen et. al. (2016)
Liquid-liquid extraction	Less time of extraction, low cost	Low extraction efficiency, large use of organic solvent	Chen et. al. (2015)

In order to overcome these difficulties and to meet the increasing demand of essential oil, microwave based advanced technique has been developed (Ferhat et. al. 2007). Nowadays, this conventional technique pay more attention on shortened Extraction time, safe, energy conserving process, reduced organic solvent consumption and preventing pollution.

The aim of present work was to isolate the peppermint oil from three techniques- solvent free microwave extraction, microwave assisted hydrodistillation and hydrodistillation on the various combination of operating parameters. This study was to evaluate oven heating effect and different operating parameter - (Microwave power, Extraction time, liquid to raw material ratio) on the quality and quantity of extracted mentha oil. Furthermore, extraction through proposed advanced technique was compared with traditional hydrodistillation method in term of efficiency, time consumption, energy consumption.

To the best of our Observation, there is no former report on the analysis of peppermint essential oil obtained by MAHD and SFME and compared it to the conventional hydro-distillation method on the proposed parameters.

## MATERIALS AND METHODS

### Materials and Chemicals

Peppermint leaves used in this experiment were hand-harvested from farm of Daliyanpur situated near Lucknow in U. P. India. The plant was freshly cut, 90-120 cm from root in the morning of April 29, 2017. To keep the raw material fresh, the leaves were chopped into small pieces with length and width of 3-6 mm and stored in a 4.7° C refrigerator before use. Distilled water and anhydrous magnesium sulfate were purchased from Nath chemicals Pvt. Ltd.

### Estimation of Moisture Content in Leaves

A sample of 10.12 gram of leaves was used for the calculation of moisture %. After weighing the sample, it was kept in hot air oven for drying at 50°C for 8 hours. The weight of sample remained after 8 hour of drying was 2.23 gram. The formula used for estimation of % moisture-

$$\text{Moisture \%} = \frac{G_i - G_f}{G_i} \times 100$$

Whereas,

$G_i$  = Initial weight of the sample (g)

$G_f$  = Final oven dried weight of the sample (g)

### Extraction Procedure

The isolation of volatile fraction from mentha piperita L. was done by using three methods for comparison on the various parameters.

### Isolation by MAHD

MAHD operation was performed by establishing the connection between the modified oven (model-Samsung MW71E) and Clevenger apparatus as shown in figure. The oven used as a heating source has adjustable power range up to 800W with an irradiation frequency of 2450MHz with inner cavity of 306mm x 211mm x 320mm. The purpose of this establishment is to perform the extraction of peppermint oil from leaves by utilizing water as the extracting solvent. Fresh leaves were fed into 1 L round bottom flask of borosilicate glass with water in different ratio. Extraction procedure was operated at different water to leaves ratio (4:1, 6:1, 8:1 and 10:1) at 250W for various duration of time 30, 60, 90, and 120 min. This process was performed to find out the maximum yield ratio. Similar ratio was investigated at 200, 250 and 300 W for same interval of time. Initially the rate of extraction was higher, but gradually decreases with time. Oil is collected till the last drop and left for 30min in separating funnel and then oil is separated and anhydrous magnesium sulfate was used to remove water content present in oil to make it pure. Finally oil was store and kept at 4.7° C till the GC/MS analysis.



Figure 2: MAHD Apparatus

### Isolation by SFME

Solvent free extraction method was operated in similar fashion as done in MAHD but it was solvent free, means extraction was done in the absence of solvent. In this, extraction was performed at 50, 100, and 150 gram of raw material. The process was differing as change in cell structure, because of electromagnetic waves causes the extraction which allows the essential oil to run out from the leave. Extraction from leaves was done by placing 100 grams of leaves in 1000mL of round bottom flask with no solvent and kept in oven for providing heat. The oven was operated at 200W, 250W and 300W for different extraction time (30, 60, 90 and 120 min). It was obtained as a green technique to the environment as there was no residue of waste water. The excess water was removed by drying over anhydrous magnesium sulfate in extracted oil. Pure oil was collected in the vials and then stored at 4.7° C for GC-MS analysis.

### Isolation by Traditional Hydro-Distillation

Hydro-distillation process was conducted by Clevenger apparatus of 34/35 size with heating mantle as heating source of 1 L capacity. In this procedure, the experiment was performed at 60° C for 6:1, 8:1, 10:1 and 12:1 ratio of water to leaves in the different period of time interval i.e. 30, 60, 90, 120, 150, 180, 210, 240, 270 min. Experiment was operated at atmospheric pressure. The extracted oil was collected in the separating funnel and after collecting, water in peppermint oil was removed by drying over anhydrous magnesium sulfate. Pure oil was then stored in the vials and put in refrigerator at 4.7° C.



Figure.3: HD Apparatus

### Analysis of Oil

#### Estimation of Oil Yield

Yield of pure oil was determined with the given equation<sup>[7]</sup> to investigate the performance of above method:

$$\text{Yield (\%)} = \frac{\text{essential oil (g)}}{\text{sample weight (g)}} \times 100$$

### Analytical Identification with GC-MS Technique

This technique is a combination of gas chromatography and mass spectrometry, which identify the presence of volatile compound. Model (Agilent Technologies-5975B) with HP-5MS fused silica non-polar capillary column equipped in GC system was used to determine the compound fraction. Dimension of capillary column used 30m×0.25mm×0.25µm. Helium (carrier gas) was used with even flow rate of ±1.5 ml/min. Separation of molecules was based on their relative affinity toward stationary phase of the column. Travelling time for each molecule was different known by retention time. Therefore eluted at different time from the column, captured by mass spectrometry, ionized into mass to charge ratio, detect ionized compound separately. 220° C and 250° C was injector and detector temperature respectively. Oven was operated at 55° C (hold 5 min) then increased with the rate of 4.5° C/min to 185° C (hold 5 min) and then at last to 230° C at rate of 8° C/min. The 1 µL sample was injected into system with the split ratio of 120:1.70 eV energy of system was used to detect the spectra of component. Peak area (%) was equivalent to % value of component present in the mentha oil.

### Energy Consumption Calculation

The energy required to achieve the complete extraction was calculated with the given below equation. Power consumption by heating source was basis to calculate the energy required from all three method.

$$E \text{ (KWh/day)} = \frac{P(W) \times t \left( \frac{h}{day} \right)}{1000 \left( \frac{W}{KW} \right)}$$

E= Energy Consumption.

P= Microwave power

## RESULTS AND DISCUSSIONS

In this work, impact of the various factors tested- extraction technique, solvents used, time, and solvent-to-sample ratio, at various levels, on the extraction yield was performed. SFME, MAHD and HD were compared on the distinct parameters for achieving higher yield.

### Analysis of Moisture Content

The moisture content of the *Mentha piperita* L. was measured before use, which was 77%±3%.

### Investigating the Yield from Different Techniques

#### 1SFME

In SFME, the yield (1.02%) obtained from 100 gram of leaves was maximum at 250W in 90 min of time. It was noted that initially extraction rate was higher till 90 min, but with course of time there was no notable change in the oil extraction. It was observed from the laboratory work, that appropriate amount of heat was required to rupture the cell so maximum amount of oil can be extracted without the any thermal degradation with maintained quality. Similarlyby changing the load (50gm and 150gm) at constant 250watt the yield was 0.86% in 60 min and 0.98% in 90 min respectively.

#### MAHD

Result shows that yield 0.98% (w/w) obtained from MAHD was maximum, when 50 gram of constant load was taken in the ratio 1:8 of plantmatter (load) to distilled water. Analyses were also made on 1:4, 1:10 ratio at 250 watt for 90



min which yield 0.65%, 0.72%, 0.98% and 0.90% respectively. The study shows that proper quantity of extraction solvent must be taken in order to maximize the yield. Higher ratio of water may cause unnecessary waste and complexation and lower can cause the incomplete extraction. It was observed that when power was increased from 200W to 250W, the yield of oil increases(0.62% to 0.98%) while further increase to 300W, yield decreased to 0.84% in 90min for similar ratio. This shows that high microwave power has negative impact, which might tend to overheat the product, and reduces the yield.

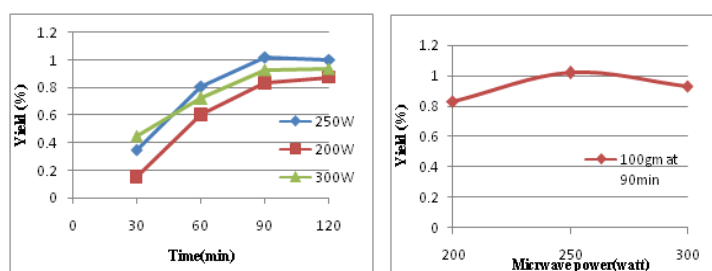


Figure 4: Effect of Extraction Time and Watt on the Yield of Oil from SFME Method on 100 gm Sample

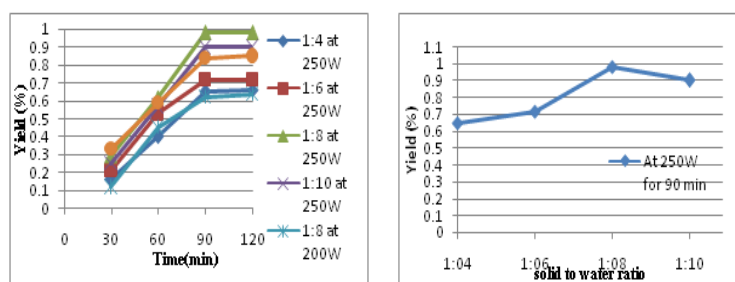


Figure.5: Effect of Different Variables on the Yield of Oil in MAHD Method

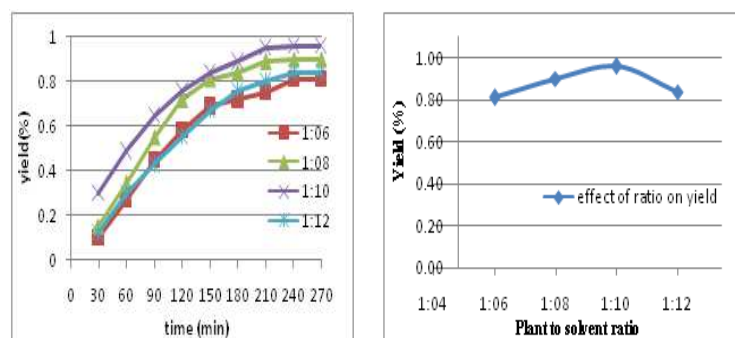


Figure.6: Effect of Extraction Time and Raw Material Ratio on the Yield of Oil in HD Method

## HD

In this method, experiment was performed at different ratio- 1:06, 1:08, 1:10 and 1:12 at constant temperature of 60°C. In this study, it was found that combination 1:10 provide maximum yield 0.96%. An increasing rate of oil yield was achieved from 30-240 min, after this there was no significant change in yield. This prevails that MAHD required less solvent to extract complete peppermint oil from leaves as compared to HD method.

### Contrasting Between the Efficiency of Above Technique

In this study, we compared the ability of MAHD, SFME and traditional hydrodistillation to extract the peppermint essential oil. SFME and MAHD both were observed as a faster process and consumed low energy as comparison to hydrodistillation method. The ultimate yields of essential oil obtained by SFME were high in comparison to MAHD and HD method. The ultimate yield obtained by SFME was 1.02% in 90 min, by MAHD method 0.98% both in 90 min and from HD method 0.96% that obtained from 4.5 hour. The extraction is clearly beneficial and profitable for the proposed SFME method in terms of time and energy. It provides substantial energy saving with the quality of extracted oil. The energy calculated, required to perform the SFME and MAHD was 0.375KWh as in comparison with HD method. The energy calculated necessary to perform the HD was 1.0125KWh and with lesser yield than previous two. Based on above results, SFME technique shows benefit over other techniques: required less extraction time, energy saver, solvent free extraction and less ejection of CO<sub>2</sub> (Eco-Friendly). These makes the SFME better alternative over other method for worth extraction.

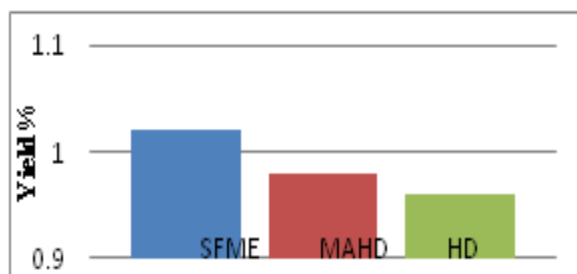


Figure.7: Comparison between Three Methods

### Compound Identification by GC-MS

Table 2 illustrates the fraction of compound present in the mentha oil extracted from SFME, MAHD and HD method. Total of 30 components were identified by SFME's extracted oil and 29 compounds by MAHD method and 31 compounds by HD method. The oil extracted from all three methods were dominated by oxygenated hydrocarbon and terpenoids. Menthol, menthone, limonene, menthofuran, menthene, isopulegol, 3- paramenthene and many more components were recognized by GC-MS in all three methods with more and less composition. Results show that menthol was the major key component in peppermint oil in all three methods.

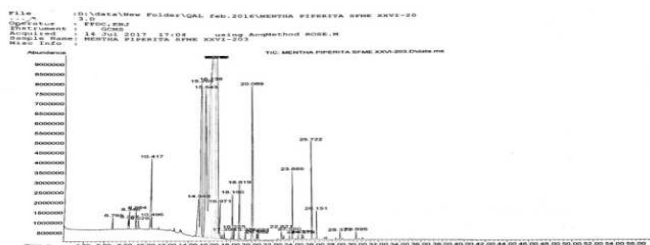


Figure. 8: Gc-MS Spectra of Oil Extracted from SFME



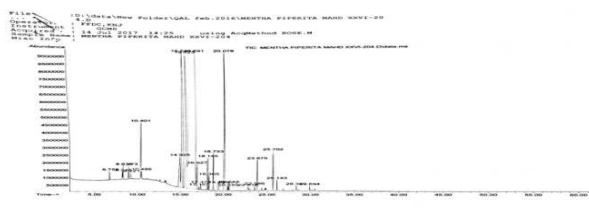


Figure.9: Gc-MS Spectra of Oil Extracted from MAHD



Figure.10: Gc-MS Spectra of Oil Extracted from HD

Table 2: Comparison of Constituents in Extracted Essential Oil in Three Methods

		Extraction Method						
Sr. No	Compounds	HD		MAHD		SFME		CAS
		RT	% A	RT	% A	RT	% A	
1	$\alpha$ -pinene	6.766	0.20	6.749	0.24	6.766	0.22	000080-56-8
2	$\beta$ -Phellandrene	8.255	0.18	-	-	8.254	0.19	000555-10-2
3	$\beta$ -pinene	8.341	0.34	8.332	0.31	8.349	0.34	000127-91-3
4	Myrcene	8.986	0.33	8.977	0.32	8.986	0.37	000123-35-3
5	3-Octanol	9.227	0.16	9.218	0.17	9.226	0.20	000589-98-0
6	Limonene	10.414	1.94	10.405	1.76	10.414	1.95	000138-86-3
7	1,8-cineole	10.491	0.17	10.482	0.18	10.500	0.20	000470-82-6
8	Isopulegol	10.491	2.19	14.921	2.14	14.947	2.35	121468-66-4
9	Para-Menthone	15.248	9.52	15.239	9.25	15.265	9.45	000089-80-5
10	Para-Menthone	15.626	9.82	15.626	9.91	15.644	9.33	000089-80-5
11	Menthol	16.791	64.30	16.693	63.90	16.779	61.66	000089-78-1
12	$\alpha$ -terpineol	16.925	0.69	16.925	0.74	16.968	0.80	000098-55-5
13	Octanol Acetate	17.373	0.14	17.166	0.14	17.898	0.14	000112-14-1
14	3z-Hexenyl Butanoate	18.147	0.85	-	-	24.418	0.91	010032-15-2
15	Pulegone	18.302	0.29	18.302	0.39	18.327	0.22	000089-82-7
16	Piperitone	18.792	1.09	18.792	0.90	18.818	1.14	000089-81-6
17	N-Decanol	19.351	0.28	-	-	19.377	0.29	000112-30-1
18	3-Para menthene	20.065	4.35	20.082	0.13	20.091	5.06	000500-00-5
19	3-Para menthene	20.469	0.09	20.469	5.56	20.091	0.13	000500-00-5
20	Nonanyl Acetate	20.555	0.10	20.555	0.08	20.564	0.09	000143-13-5
21	Isopulegyl Acetate	20.616	0.12	20.616	0.16	20.624	0.14	109010-11-9
22	$\beta$ -Bourbonene	22.818	0.02	22.818	0.12	22.818	0.25	005208-59-3
23	Decyl Acetate	23.592	0.15	23.583	0.11	23.592	0.14	000112-17-4
24	E-Caryophyllene	23.876	0.84	23.876	0.73	23.893	1.36	000087-44-5
25	Hexyl 2-menthyl Butanoate	24.418	0.09	-	-	24.418	0.09	010032-15-2
26	Piperitone	24.770	0.07	-	-	24.779	0.10	000089-81-6
27	D- Germacrene	25.699	0.94	25.699	0.89	25.725	1.96	023986-74-5
28	Bicyclogermacrene	26.147	0.31	-	-	26.155	0.09	024703-35-3

Table 2: Contd.,								
29	Delta-Amorphene	28.375	0.12	-	-	29.897	1.96	189165-79-5
30	3z-Hexenyl phenyl Acetate	29.897	0.22	-	-		0.16	042436-07-7
31	Delta-Amorphene	30.499	0.13	-	-		-	189165-79-5

## CONCLUSIONS

In this study, solvent free microwave extraction and microwave assisted hydro distillation have been compared with the conventional hydrodistillation method for the extraction of essential oil from the aerial parts of *Mentha Piperita* L. on the above parameters. This microwave extraction method offers important advantages over traditional methods such as hydrodistillation. This study also revealed that these two techniques consumed less energy as comparison to HD method. SFME process is more effective and provide higher yield in comparison with other two methods. This indicated that advance technology, MAHD and SFME has better potentially that other conventional method HD. SFME had become rising technology that requires a shorter extraction time to produce essential oil compared to other method. The chemical constituents present in the peppermint essential oil by all three methods show no significant difference in the quantity of the major compounds and also most of minor compounds present in the extracted essential oil. The energy consumption in these advance technologies requires less with comparison to conventional HD method.

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